developmental time window to initiate lateral compartment differentiation. In the absence of FGF20, lateral compartment cells remain undifferentiated and postmitotic, and unresponsive to Notch-dependent lateral inhibition. The requirement in mice of FGF20 for OC development and hearing, and the lack of other severe phenotypes in the absence of FGF20, suggest that human FGF20 may be a candidate hereditary deafness gene.

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Program/Abstract # 246
The role of Hes/Hey genes in the sensory development of the chicken inner ear
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The Notch pathway plays an essential role in the specification of the prosensory patches and in the determination of hair cells and neurons. The prosensory function of Notch is mediated by Jagged1 (Jag1), which restricts Sox2 expression to the prosensory patches via a mechanism of lateral induction that propagates the Notch signal within the prosensory domains. However, it is not known what couples Notch signaling to lateral induction and Sox2 expression. We have explored the expression patterns of Hes/Hey genes as potential candidates for downstream targets of Notch in the ear. The results show that Hey1 corresponds well with Jag1 expression in the prosensory patches. Hey1 expression is homogeneous within the prosensory patches and parallels lateral induction. On the contrary, Hes5 expression is speckled and delayed with respect to Hey1. It overlaps with Dl1 expression, and both parallel lateral inhibition during neurogenesis and hair cell determination. Hes1 is expressed weakly in sensory patches and Hey2 is mainly expressed in the periotic mesenchyme. The expression of both Hey1 and Hes5 depends on Notch activation and is abolished by DAPT. The forced expression of hJag1 in the otic cup induces Hey1, but not Hes5. After sensory specification, however, hJag1 is unable to induce Hey1 expression outside the sensory domains, suggesting that the competence of the otic epithelium to respond to Notch becomes restricted throughout development. The effect of hJag1 on Hey1 was mimicked by hJag2 but not by cDl1 overexpression. The results suggest that Hey1 is a good candidate to mediate the prosensory function of Notch. Moreover different Notch ligands are associated with different targets and modes of action of Notch.

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Program/Abstract # 247
Sox2 and Ngn1 regulate the neurogenic fate in the developing inner ear
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In the central nervous system (CNS), Sry-related HMG-box 2 (Sox2) is thought to inhibit neurogenesis by keeping neuronal progenitors in an undifferentiated state but its action is counteracted by Ngn2 (Neurogenin 2) to promote neurogenesis. In contrast, over-expression of Sox2 has been shown to promote neurogenesis in mouse cochlear explants. In the developing inner ear, Sox2 is expressed in the neural-sensory competent domain (NSC), but its expression is down-regulated in the neuroblasts that delaminate from the NSC domain to form the cochleovestibular ganglion (CVG). To investigate the role of Sox2 in neurogenesis of the inner ear, we over-expressed Sox2 in the developing chicken inner ears in ovo. Our results indicate that ectopic Sox2 readily induces Neurogenin 1 (Ngn1) expression, an important gene required for the neurogenic fate of the inner ear. Nevertheless, neurogenesis fails to proceed based on the lack of Neurod1 up-regulation and as a result, the size of CVG is reduced. Over-expression of Ngn1 is capable of up-regulation of Neurod1 and causes ectopic neuroblast formation in the otic cup. Similar increases in neurogenesis are obtained with over-expression of Neurod1. Based on these results, we hypothesize that Sox2 is normally involved in initiating neurogenesis by up-regulating Ngn1. The up-regulated Ngn1, in turn, down-regulates Sox2 expression in order for neurogenesis to proceed and Neurod1 to be up-regulated. We provide evidence that Ngn1 inhibits Sox2 expression at the transcriptional level. The inability of Ngn1 to repress the transcription of exogenous Sox2 leads to the failure of neurogenesis in over-expressed Sox2 specimens.

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Program/Abstract # 248
Fate-mapping the vestibular neurogenic region in the developing chicken otic cup using lipophilic dyes
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During inner ear development, precursors of auditory and vestibular neurons delaminate from a neuro-sensory competent domain of the otic cup. This domain eventually gives rise to sensory organs. Previous fate-mapping studies in chicken suggest that precursors of vestibular and auditory neurons are regionally segregated as early as the otic placode stage. This regional organization of the neurogenic region may dictate the type of sensory organs that subsequently form. Here, we show that by the otocyst stage, the Neurod-positive neurogenic domain is delineated by a Crabp1-positive lateral region and a Pax2-positive medial region, which presumably correspond to the areas that give rise to vestibular and auditory neurons, respectively. To verify the neurogenic identity of these two regions, we fate-mapped the presumed vestibular-neurogenic region by focal injections of lipophilic dyes along the lateral edge of the Neurod-positive domain of the otic cup, and follow the labeled cells at the otocyst stage and a later stage when the neurons and sensory organs are differentiated. Our results indicate that labeled cells from the Crabp1-positive lateral region of the neurogenic domain in the otocyst give rise to mostly vestibular neurons. In contrast, labeled cells in a region which weakly expresses both Crabp1 and Pax2 give rise to both vestibular and auditory neurons. Despite the differences in the resulting labeled neurons, the entire lateral edge of the neurogenic domain in the otic cup only label cells within the vestibular sensory organs. The results are consistent with the notion that neuronal fates and subsequent sensory organ types in the inner ear are related.

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Program/Abstract # 249
The role of Hh signaling and proneural genes in otic neurosensory development
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The Role of Hh Signaling and Proneural Genes in Otic Neurosensory Development D. Sapède, S. Dyballa and C. Pujades Department of Experimental and Health Sciences, Universitat Pompeu Fabra, Barcelona, Spain The inner ear is responsible for the perception of motion and sound

in vertebrates. Its functional unit, the sensory patch, contains mechanosensory hair cells innervated by sensory neurons of the vestibular and acoustic ganglia that project to the corresponding nuclei in the brainstem. How hair cells develop at specific positions, and how otic neurons are sorted to specifically innervate each endorgan and to convey the extracted information to the hindbrain is not completely understood yet. In our previous work, we studied how, when and where the formation of first-order neurons and their target hair cells takes place. We showed that the Hh pathway is crucial in coordinating the production of hair cells in the posterior macula (PM), and in the formation of its specific innervation, underlying the importance of Hh pathway in the de novo formation of a fully functional posterior sensory patch. Nevertheless, how Hh signaling is involved in defining a PM-specific identity is still unknown. One interesting question that this work highlights is how Hh confers saccular (PM) identity. Hh signaling might direct the development of both neuronal and sensory progenitors within the posteromedial otic domain. This would suggest that there is a common pool of progenitors for saccular hair cells and neurons located in the postero-medial territory in the otic epithelium. We want to address how the generation of neurons and sensory cells in this territory is coordinated – focusing on the role of proneural genes – and whether there is a common progenitor that responds to different spatial and temporal cues. Expression analysis of proneural genes in this otic territory shows that expression domains of bHLH transcription factors for neurons and sensory cells partially overlap within the posteromedial otic domain. In addition, functional experiments with neurog1 and neuroD genes suggest that neurog1 defines a posteromedial field of progenitors with competence to form PM hair cells, supporting the idea of a common pool of neurosensory precursors in the zebrafish inner ear. DS was a recipient of a postdoctoral JdC contract from MICINN (Spain) and SD is supported by a predoctoral FI fellowship from AGAUR (Generalitat de Catalunya). This work has been funded by the grant BFU2009-07010 from MICINN (Spain) to CP.

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Program/Abstract # 250
Exploring the function of hair-cell-enriched microRNAs in vitro and in vivo

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To detect sound and sense balance, animals rely on a specialized group of cells known as mechanosensory hair cells (HCs). HCs arise from the same group of progenitors as their supporting cell neighbors. The bHLH transcription factor, Atoh1, is both necessary and sufficient for HC fate acquisition, while a family of HC-enriched miRNAs, the miR-183 family, can influence HC numbers, morphology and progression of differentiation. These and other miRNAs may function to repress genes required to maintain the progenitor status, to adopt the alternative supporting cell fate, or both. If so, then the simultaneous delivery of both Atoh1 and specific miRNAs should enhance HC development. We are testing this in two ways: by validating predicted target genes of miR-182 (a miR-183 family member) in vitro and by creating gene transfer vectors that force expression of both Atoh1 and selected miRNA genes in vivo. In vitro luciferase assays show that miR-182 targets at least 14 3'UTRs, including some HC transcripts (e.g., Myosin1C and Myrip, a Rab effector that recruits myosins). For gene transfer, a bifunctional cassette was created that houses the miRNA-183 family in an artificial intron upstream of Atoh1 fused to a HA tag, all driven with the EF1α promoter. To date, the function of vector-transduced Atoh1 and the miR-183 family has been confirmed by in vitro luciferase reporter assays. For in vivo studies, bifunctional vectors are electroporated into E3 chicken otocysts and analyzed 2 days later. In vivo experiments confirm that Atoh1 alone can generate ectopic HCs. Future research is focused on analyzing otocysts injected with the entire bifunctional cassette as well as assessing what impact these miRNAs alone have on HC development.

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Program/Abstract # 251
Specification of sensory progenitors: Towards a gene regulatory network

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In the head, sense organs and sensory ganglia largely arise from the ectoderm outside of the central nervous system, the sensory placodes. During development they are derived from a pool of multipotent progenitor cells that are set aside at neural plate stages. To uncover molecular mechanisms controlling their specification we have identified the signaling pathways that induce sensory fate in naïve ectoderm as well as the transcription factors that mediate their action. Members of the Six and Eya gene families play an important role and in addition we have identified new genes that may act up-stream, down-stream or in parallel to these factors to impart sensory progenitor identity. Current experiments aim to determine their genetic hierarchy and interaction and we present a gene regulatory network that models sensory progenitor specification and diversification.

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Program/Abstract # 252
The role of the zinc-finger transcription factor Sp8 in the establishment/maintenance of the dorsal lateral ganglionic eminence (dLGE)

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The embryonic lateral ganglionic eminence (LGE) is an important progenitor domain that gives rise to olfactory bulb interneurons and striatal projection neurons. Recent work has suggested that these two neuronal subtypes arise from distinct progenitor populations within the LGE. The dorsal (d)LGE is proposed to give rise to olfactory bulb interneurons while the ventral (v)LGE generates striatal projection neurons. Previous work has shown that the zinc-finger transcription factor Sp8 marks the dLGE subventricular zone (SVZ) as well as the postnatal SVZ. Additionally, conditional deletion of Sp8 in the ganglionic eminences results in the reduction of olfactory bulb interneuron subtypes. However, it remains unclear whether Sp8 plays an active role in the establishment and/or maintenance of the dLGE SVZ. To study the role of Sp8 in defining the dLGE we have taken a gain-of-function approach. We generated a tetO-Sp8-IRES-EGFP line and expanded the expression domain of Sp8 throughout the LGE SVZ using a recently developed Dlx5/6-tTa mouse line. Our results suggest that expansion of the Sp8 domain results in the downregulation of vLGE markers such as Islet-1 at late embryonic and early postnatal stages. Furthermore, the overexpression of Sp8 leads to an enlarged SVZ/Rostral Migratory Stream (RMS) in the postnatal brain and a concomitant reduction in striatal size. Doxycline treatment of the pregnant females harboring Sp8 overexpressing embryos, delayed activation of the Sp8 transgene until E15 and did not result in respecification of vLGE to dLGE fates that was seen at early time points. Our results therefore support a role for Sp8 in establishing/maintaining dLGE identity within the LGE SVZ at early embryonic stages.

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